Chapter 22

EFFECTS OF MYCOPHAGOUS COLLEMBOLA ON *RHIZOCTONIA SOLANI* AND COTTON-SEEDLING DISEASE

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Abstract

Collembola in the genera *Proisotoma* and *Onychiurus* are more abundant in the rhizosphere of cotton (*Gossypium hirsutum*) than in root-free soils in Alabama. In laboratory feeding tests, mixed populations of *P. minuta* and *O. encarpatus* rapidly destroyed young cultures of *Rhizoctonia solani* growing on water agar from potato-dextrose agar discs and oatgrain substrates. The insects were not attracted to sterile organic matter in the same dishes. Both species of Collembola, when tested separately, showed a preference for *Rh. solani* over *Trichoderma harzianum*, exploiting the latter only in the early growth stage before sporulation. Experiments conducted in large glass tubes of sterilized sandy loam, artificially infested with *Rhizoctonia* mycelium and Collembola prior to planting disinfested cotton seed, showed a reduction in disease severity. Subsequent greenhouse tests with non-sterilized field soil, naturally infested with *Rh. solani* or supplemented with the pathogen on ground oats (0.02 g/kg soil), revealed a control benefit attributed to added Collembola in populations of 1000 and 2000/kg soil. Percentage seedling emergence was significantly increased, disease severity was reduced, and plant growth (root + shoot length) increased. Results of this study suggest the need of further studies on mycophagous microarthropods, especially Collembola, in ecological investigations relating to rhizosphere phenomena and root disease.

Introduction

Interactions among components of the soil microflora and their potential relation to biological control of root-infecting fungi have been studied for many years (Baker and Cook, 1974). Yet, feeding of the soil fauna upon fungi has been given little attention as a phenomenon under the term antagonism. Nearly 50 years ago, Starkey (1929) suggested that "the attraction of phytophagous insects and worms about roots may eventually lead to influences upon the micro-organisms". Kevan (1965), at the First International Symposium on Factors Determining the Behaviour of Plant Pathogens in Soil, discussed the nature and biology of the soil fauna in relation to the microflora. He recognized that populations of soil microarthropods in particular may be substantial, the Acarina and Collembola being the most abundant of soil-inhabiting animals exclusive of nematodes and protozoa.

The order Collembola (springtails) is comprised of minute insects, usually 1-3 mm in length, many species of which inhabit the top 10-15 cm of soil. They are especially abundant in grasslands and forest soils, while numbers generally are considered to be small in cultivated lands of low organic matter content. Wallwork (1976) and Wiggins (1976) have provided pertinent information and references to the distribution of Collembola and the effects of various agricultural practices on their activities. Some species are predominantly mycophagous, as evidenced by observed feeding habits and mycelia and spores found in the gut contents (Poole, 1959; Christiansen, 1964; Anderson and Healy, 1972; Christen, 1975). Coleman and McGinnis (1970) buried radioisotope-labelled mycelium of *Geotrichum* sp. in field soil and found that most of this food source was consumed by Collembola rather than by mites.

Two mycophagous species of Collembola, *Proisotoma minuta* Tullberg (Isotomidae) and *Onychiurus encarpatus* Denis (Poduridae), were predominant in cotton-field soils of Alabama, and were invariably more abundant in cotton-seedling rhizosphere than they were distant from roots (Wiggins *et al.*, 1979). Supplementary inorganic fertilization also induced higher numbers. Based on further studies of the interaction between cotton-rhizosphere Collembola and the microflora, Wiggins and Curl (1979) postulated a possible dual role for these insects that might be disease-related, i.e. transmission of spores to the rhizosphere, and reduction of inoculum density by feeding on pathogen mycelia.

The present investigation was designed to determine the destructive feeding capacity of two species of Collembola upon the mycelium of *Rhizoctonia solani* Kühn and, subsequently, to assess their influence on pre- and post-emergence damping-off of cotton in sterilized and non-sterilized field soil.

Material and methods

The species of Collembola used throughout this study, *P. minuta* and *O. encarpatus*, were extracted from the rhizosphere of 6-week-old cotton seedlings by the modified Tullgren extraction system described by Wiggins and Curl (1979). The insects were maintained and reproduced on cultures of *Rh. solani* on potato-dextrose agar (PDA), since Wiggins and Curl had shown the fungus to be a favourable food source and that the insects did not transport the pathogen in sufficient quantity to initiate disease in cotton. The isolate of *Rh. solani* used in all phases of the study was obtained from diseased field cotton and was extremely virulent at low inoculum levels.

Insect feeding experiments

First, it was desirable to measure the destructive capacity of the insects upon mycelium of the *Rhizoctonia* isolate to be used later as soil inoculum. This was accomplished in a series of simple petri-dish tests.

A mycelial disc (6 mm diameter) of *Rh. solani*, taken from the periphery of a young culture on PDA, and a sterile PDA disc were placed 4.5 cm apart on the surface of water agar in a petri dish. A culture-dish of *Rh. solani* containing a mixture of the two Collembola species was inverted over a dry, short-stem funnel and tapped sharply to dislodge the insects, which fell to the centre of the water-agar dish between the two PDA discs. The insects were applied either immediately after plating the *Rhizoctonia* disc or 24 h later. Six replications were prepared and controls consisted of similar dishes without insects. Distribution patterns of the insects in relation to the *Rhizoctonia* and sterile PDA discs were recorded photographically after 24 and 48 h incubation at 25°C in darkness.

Other similar tests in the series were as follows:

1) *Rhizoctonia solani* was cultured on sterilized oats, which were then air-dried, and one infested oat grain was placed on water agar opposite a sterile oat grain; mixed Collembola were added as before.
2) Since insects in field soil are afforded many other fungal food sources, a common representative soil inhabitant, *Trichoderma harzianum* Rifai, was included in this test; a mycelial disc of each fungus was placed on water agar in a dish and insects were added.
3) The preceding test was repeated, except that *Rh. solani* and *T. harzianum* on infested oats were applied and the two insect species within a 2.5 mm diameter area around each substrate were counted under a dissecting microscope and recorded as percentage of the total number present. All insects outside these specific areas were recorded as occupying a "neutral" zone. Counting was facilitated by first holding the dishes in a freezer for 1 h at -20°C to stop insect movements they are cold-tolerant and not killed by this treatment.

Laboratory experiments in sterilized soil with cotton

Experiments were conducted in glass tubes of sterilized soil artificially infested with measured quantities of *Rh. solani* inoculum and varied numbers of *P. minuta* and *O. encarpatus*; surface-disinfested, pre-germinated cotton seed (*Gossypium hirsutum* L., 'Stoneville 213') were then planted in the tubes. Cotton seed used in all tests were acid-delinted and had not been treated with a pesticide.

The soil used was a fertile Dothan sandy loam from a field plot which had received the same annual fertilization treatments and a 3-year rotation of cotton, soybean and corn for 11 years. Annual fertilization provided nitrogen, phosphorus and potassium at rates of 135, 33 and 83 kg/ha plus lime and minor elements. The pH of the soil when collected was 6.0. A 2-mm sieve fraction of thoroughly mixed soil was adjusted to 12% moisture (w/w) and sterilized in bulk by autoclaving for 1½ h at 120°C. Large test tubes, 34 mm I.D. x 20 cm, equipped with cotton-plugged extension sections (total length of growth tubes 22.5 cm), also were autoclaved.

Rhizoctonia solani was cultured for 7 days in 250-ml flasks with 30 ml of potato-dextrose broth. The mycelial mats were washed by centrifugation in sterile demineralized water, then chopped for 1 min in a "semi-micro" blender. A desired quantity (wet-weight) of the chopped mycelium was then suspended in sterile water and added aseptically to half of the bulk of autoclaved soil and thoroughly mixed in; the other half of soil was not inoculated. The soil was then dispensed through a sterile funnel into the growth tubes, about 250 g moist soil per tube, and the tubes were tamped lightly on a table top for uniform packing of the soil. Four treatments were prepared: sterile soil only (SS), sterile soil + added Collembola (SS+CO), sterile soil + *Rh. solani* (SS+RS) and sterile soil + *Rh. solani* + Collembola (SS+RS+CO).

Predominantly mature insects of the two species, mixed in approximately equal numbers, were shaken from rearing dishes into a sterile glass funnel that directed them to the soil surface in the growth tubes. They were allowed 48 h to become established in the new environment before cotton seeds were planted. Cotton seeds were surface-sterilized for 30 min in a 3:1 solution of 2% sodium hypochlorite and 70% alcohol plus a small amount of Tween-20 wetting agent, then washed in sterile water and placed on Czapek's nutrient agar in 1.5 x 14 cm culture dishes. When the radicles were 5-10 mm long, two uncontaminated seedlings were transplanted to each growth tube. The tubes were wrapped with brown paper to keep soil and roots in darkness. Treatments were randomized in racks (see Figure 2) under combined incandescent and fluorescent lights (about 6 klux) automatically controlled for 10 h exposure alternating with a 14 h dark period. The light-period air temperature was constant at 27°C and the dark-period at 25°C.

Four tests in sterilized soil, as described above, were conducted with two inoculum densities of *Rh. solani* and varied numbers of Collembola applied. Combinations of *Rhizoctonia* inoculum (g wet mycelium / kg soil) and estimated numbers of insects (per tube) were: 1) 0.05 g + 400, 2) 0.05 g + 1200, 3) 0.01 g + 600, and 4) 0.01 g + 1200. Treatments were replicated 10 times in all tests except those in 4), which were replicated 20 times.

Twelve days after planting, soil and roots were gently removed from the tubes. The roots were washed, total lengths of plants (roots and shoots) were measured, and disease severity was rated on a scale of 0-5, where 0 = healthy, 1 = slight browning of root, 2 = root consisting of ¼ dead tissue, 3 = ½ dead tissue, 4 = ¾ dead tissue and 5 = dead plant. For one test, an estimate was made of the relative concentration of *Rh. solani* in the soil of treatmeants SS+RS and SS+RS+CO. Six areas 2 cm in diameter were drawn from top to bottom, 2.5 cm apart, on the surface of five tubes of each treatment, and the number of mycelial strands crossing these areas was counted. Other observations were recorded photographically.

Greenhouse tests in non-sterilized field soil

Soil was collected to a depth of 20-25 cm during June and July from the same field plot used for laboratory tests; the plot was in corn but had been in cotton the previous season. The relative, natural infestation of the soil with *Rh. solani*, determined by a stem-trap method (Papavizas and Davey, 1962), was found to be very high as indicated by 100% colonization of buried cotton-stem sections. The soil (sandy loam), being relatively free of large pieces of organic matter, was not screened before use, and fresh soil was collected for each repeated test.

Inoculum was prepared by growing *Rh. solani* in flasks of sterilized oats wetted with a Czapek's nutrient solution. The infested oats were then air-dried and coarsely ground with a mortar and pestle. The required amount of ground oats for a given test was mixed with a small portion of soil, then thoroughly mixed into a larger bulk; the soil moisture at this time was about 6%. An equal portion of the non-sterilized soil was not supplemented with *Rh. solani*, but left only naturally infested. The soil was dispensed in 900 ml quantities into 1 litre plastic pots with bottom drainage holes. The quantity of supplementary inoculum required in non-sterilized soil (NSS) to allow 20-30% cotton emergence was pre-determined by several pilot tests in laboratory growth tubes and greenhouse pots with levels ranging from 2.3 to 0.02 g ground-oat inoculum/kg soil. The lowest level (0.02 g) was most suitable and was used in the principal tests consisting of the following treatments replicated eight times: NSS, NSS+CO, NSS+RS and NSS+RS+CO. The two species of Collembola, mixed in approximately equal numbers, were added by funnel to the soil surface. Two independent tests were conducted, each with freshly collected field soil, one test involving approximately 1000 Collembola/kg and the other 2000/kg soil.

Twenty-four hours after *Rh. solani* and the Collembola had been applied, ten cotton seeds were planted in each pot and covered with a 1 cm layer of vermiculite. The treatments were completely randomized on a greenhouse bench. The soil was lightly watered at planting time and then watered uniformly as needed thereafter. Soil temperatures during the 12-day growth period ranged from 23 to 35°C.

Percentage emergence of seedlings was recorded when it was evident that maximum emergence had occurred. At termination of the test, all plants and ungerminated seeds were carefully recovered from the soil and washed; root-disease severity was rated on a scale of 0-5 representing a range of conditions from healthy to dead. Ungerminated seed and rotted hypocotyls were categorized as pre-emergence damping-off and given a rating of 5. Soil from treatments NSS and NSS+CO was assessed for *Rhizoctonia* infestation by the cotton stem-trap method previously referred to. Washed root sections from each treatment were plated on water agar containing streptomycin sulphate (100 μg/ml) and examined with a microscope after 18 h incubation at 25°C to verify the presence of *Rh. solani*.

Results

Mycophagous activities of Collembola

In the first two of a series of feeding tests, observations were recorded only photographically. Where a PDA disc of young *Rh. solani* mycelium was paired with a sterile PDA disc on the surface of water agar, added Collembola of both species aggregated almost entirely on and around the fungal disc (see Figure 1B) and showed no attraction to PDA alone. After 24 h, no surface mycelium was visible on the disc, and the insects remained within 1-2 cm of the fungal disc for 48 h and often longer, evidently grazing any hyphal regrowth. The control disc of *Rhizoctonia* without Collembola (see Figure 1A) shows normal growth at *c.* 12 h. When the fungus was allowed to grow for 20 h before application of approximately 200 insects, destruction of surface mycelium was almost complete within 2 h, leaving only a pattern of subsurface mycelium which the Collembola could not reach; they did not burrow into the agar but made many small pits in the surface in what appeared to be attempts to reach the hyphae. At this time the insects on the water agar surface had assumed a circular pattern of distribution conforming to that of the subsurface colony.

Figure 1, C and D show similar observations when *Rh.*

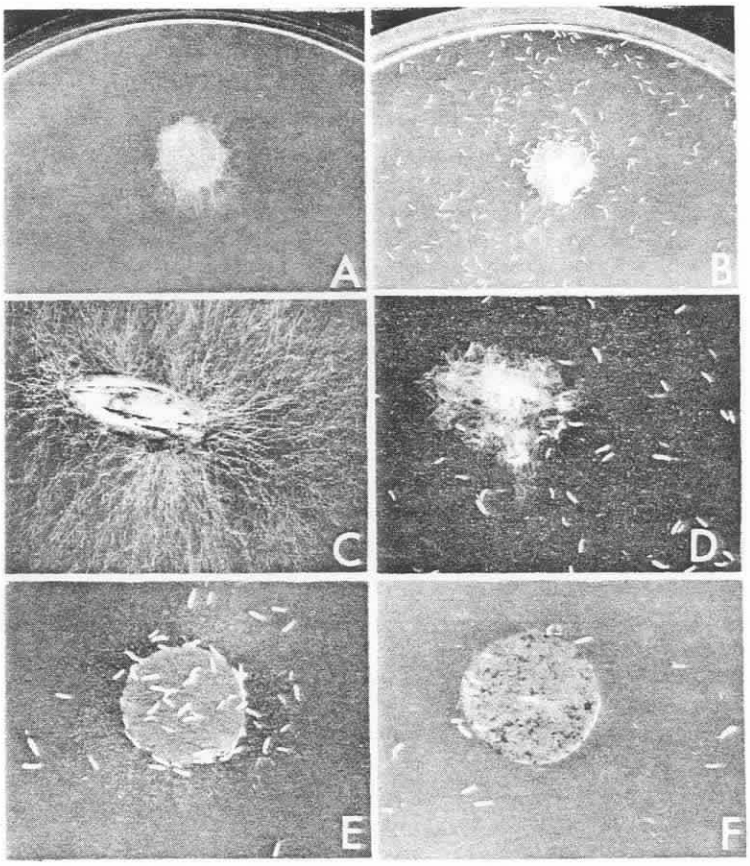

Fig. 1 Feeding of mixed species of Collembola on young cultures of fungi. A-B) *Rhizoctonia solani* on potato-dextrose agar discs without Collembola and with insects added. C-D) *Rh. solani* on oat grain without and with Collembola. E-F) *Rh. solani* (E) and *Trichoderma harzianum* (F) on PDA discs with Collembola added.

solani was applied on infested oat grains. Within a few minutes, the insects were attracted to the infested oat, destroying most of the fungus within a few hours; the sterile oat remained uninhabited except for chance encounter.

The feeding "preference" of Collembola for *Rh. solani* over *T. harzianum* was evident, whether the fungi were provided on PDA or oat substrate. If *Rh. solani* was not present, some insects fed at the growing edge of *T. harzianum* colonies, but avoided areas of sporulation. Data from three tests showed that attraction to *Rh. solani* was almost five times greater than attraction to *T. harzianum* (Table 1). Test IV in the table represents a comparison between the two Collembola species and shows that *Rh.*

solani was the preferred food source for both species, this being somewhat more evident for *O. encarpatus*. The largest number of *P. minuta* was observed in a "neutral" zone not near either fungus; yet, this species alone can destroy *Rhizoctonia* almost as rapidly as *O. encarpatus*.

TABLE 1

Comparative attraction of Collembola to young colonies of Rhizoctonia solani *and* Trichoderma harzianum *initiated from mycelial discs of PDA on the surface of water agar*

Feeding zone[a]	Percent of total population in feeding zone		
	Tests I-III[b]	Test IV	
	(mixed species)	*Proisotoma minuta*[c]	*Onychiurus encarpatus*[d]
Rhizoctonia solani	71.7	21.7	52.7
Trichoderma harzianum	14.6	8.0	19.6
Neutral	13.7	70.3	27.7

[a] Area of 2.5 mm diameter around mycelial disc; neutral refers to the remaining surface area of the entire plate.

[b] Three-test means from an average of 108 insects/dish, six dishes/test.

[c] Means from an average of 103 insects/dish.

[d] Means from an average of 169 insects/dish.

Effects of Collembola and Rhizoctonia *on cotton seedlings in sterilized soil*

Results from two tests, in which growth tubes of sterilized soil were infested with *Rh. solani* mycelium (0.05 g/kg) and two populations of Collembola, are presented in Table 2. A population of 400 insects per tube with 250 g soil (1600 /kg) did not reduce disease severity in 12-day-old cotton seedlings, but plants in sterile soil with Collembola only (SS+CO) attained an average total length (roots + shoots) that was 2.5 cm longer than plants in sterile soil without insects. Enhancement of plant growth by the presence of 1200 Collembola per tube (4800/kg soil) was evident in soil with or without added *Rh. solani* (see Figure 2). The accompanying disease index (Table 2) indicated complete protection of plants from serious *Rhizoctonia* injury where this relatively high insect population was used; the root systems were as well developed and unaffected as those in sterile soil. Reduced inoculum density attributable to insect feeding is suggested in Figure

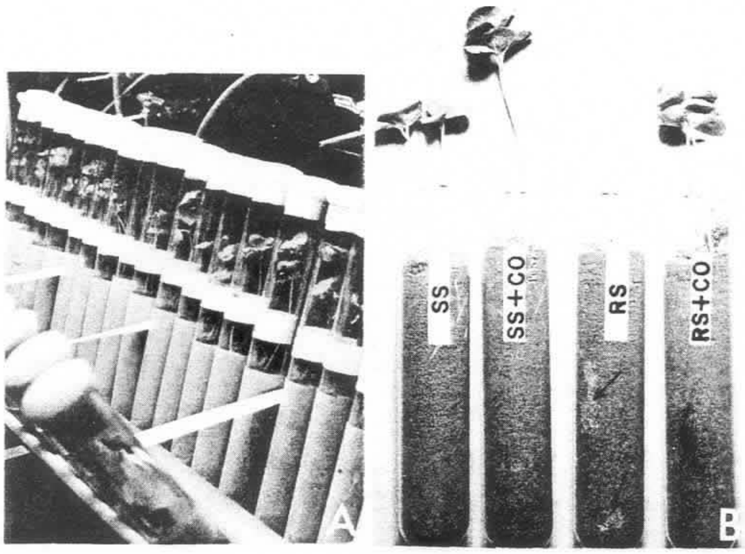

Fig. 2 Cotton seedlings in tubes containing sterilized soil, *Rhizoctonia solani*, and mixed species of Collembola. A) Growth tubes complete with extension tubes and plants arranged in racks under lights. B) Tubes with extension sections removed, showing effects of Collembola and *Rh. solani* on seedlings; note patches of *Rhizoctonia* mycelium (arrows). SS = sterile soil, CO = Collembola, RS = *Rh. solani*.

TABLE 2

Effects of Rhizoctonia solani *and two populations of Collembola on cotton seedlings in tubes of previously sterilized soil*

Treatments[a]	1600 Collembola/kg soil[b]		4800 Collembola/kg soil[c]	
	Total plant length(cm)	Disease index (0-5)	Total plant length (cm)	Disease index (0-5)
SS	23.1	0.40	22.1	0.79
SS+CO	25.6	0.10	24.3	0.42
SS+RS	3.3	5.00	16.7	3.53
SS+RS+CO	3.5	4.90	22.0	0.17

[a] SS = sterile soil, CO = Collembola, RS = *Rhizoctonia solani* (0.05 g mycelium/kg soil).

[b] Represents 400 insects of mixed species, *Proisotoma minuta* and *Onychiurus encarpatus*, per tube with two plants; data are averages from 20 plants.

[c] Represents 1200 mixed Collembola per tube.

2B. Note patches of *Rhizoctonia* visible on the soil surface within the glass tube of treatment RS and no such patches in the tube of RS+CO. This contrast was substantiated by counts of mycelial strands crossing 2-cm-diameter areas of soil surface in tubes of those treatments. The number of strands in tubes with only RS ranged from 14 to 20 per tube with an average of 16.5; in soil with RS+CO this number was reduced to the range of 4 to 12 or an average of 7.6 per tube.

When sterilized soil in tubes was infested with *Rh. solani* at a density of 0.01 g mycelium/kg, 600 insects of mixed species per tube (2400/kg soil) had a moderate control effect, reducing the disease index value from 4.55 to 3.15 (Table 3). In this test, the expected enhancement of plant growth in the SS+CO treatment was not observed, but Collembola added to the RS-infested soil negated the severe stunting of plants that occurred in SS+RS without Collembola. In RS soil with the equivalent of 4800 insects/kg, the disease severity was reduced nearly 3 index values, and improved plant growth was reflected, but not to the extent reached in soil without RS. Again, the presence of Collembola alone (SS+CO) favoured plant growth by 3.8 cm. Possible explanations for enhanced growth when only insects were added to sterile soil are offered later in the Discussion section.

TABLE 3

Effects of Rhizoctonia solani *and two populations of Collembola on cotton seedlings in tubes of previously sterilized soil*

Treatments[a]	2400 Collembola/kg soil[b]		4800 Collembola/kg soil[c]	
	Total plant length(cm)	Disease index (0-5)	Total plant length (cm)	Disease index (0-5)
SS	16.1	0.90	16.6	0.20
SS+CO	16.1	1.70	20.4	0.30
SS+RS	7.8	4.55	4.0	4.90
SS+RS+CO	16.1	3.15	9.2	2.10

[a] SS = sterile soil, CO = Collembola, RS = *Rhizoctonia solani* (0.01 g mycelium/kg soil).

[b] Represents 600 insects of mixed species, *Proisotoma minuta* and *Onychiurus encarpatus*, per tube with two plants; data are averages from 20 plants.

[c] Represents 1200 insects per growth tube with two plants; data are averages from 40 plants.

Effects on cotton seedlings in non-sterilized field soil

The data in Table 4 represent the effects of Collembola and either natural or supplementary inoculum of *Rh. solani* on cotton seedlings in pots of non-sterilized soil. Whether the soil was infested with 1000 or 2000 insects/kg, the beneficial effect on percentage emergence was striking in both naturally infested soil and non-sterilized soil supplemented with *Rh. solani* at a rate of 0.02 g infested oats/kg. A population of 1000 Collembola of the two mixed species induces a 25% increase in seedling emergence in natural field soil and a 27.5% increase in *Rh. solani*-amended soil. Comparable increases in soil with twice that number of insects were 20% to 40%; although the 20% difference between NSS (75%) and NSS+CO (95%) was not statistically significant (P = 0.05), repeated tests consistently showed a trend of increased emergence. Figure 3B shows the protective benefit of Collembola added to naturally RS-infected field soil as compared with soil not supplemented with Collembola (Figure 3A). The contrasting difference in plant density (stand) between NSS with supplementary RS (see Figure 3C) and the same treatment with added Collembola (see Figure 3D) is quite evident. Plants in pots with either 1000 or 2000 insects/kg soil consistently appeared

TABLE 4

Effects of two populations of Collembola and supplementary inoculum of Rhizoctonia solani *on cotton seedlings in pots of non-sterilized field soil*

Treatments	1000 Collembola/kg soil			2000 Collembola/kg soil		
	Emergence (%)	Total plant length (cm)	Disease index (0-5)	Emergence (%)	Total plant length (cm)	Disease index (0-5)
NSS	66.2 b	22.0 a	2.34 c	75.0 ab	20.2 a	2.25 b
NSS+CO	91.2 a	23.3 b	0.94 d	95.0 a	23.0 b	0.90 c
NSS+RS	47.5 c	--	4.65 a	48.3 c	--	4.10 a
NSS+RS+CO	75.0 b	--	3.51 b	88.3 ab	--	2.20 b

NSS = non-sterile soil, CO = Collembola, RS = *Rhizoctonia solani* (0.02 g infested oats/kg soil); 10 cotton seeds were planted per pot, eight pots per treatment. Collembola were mixed species, *Proisotoma minuta* and *Onychiurus encarpatus*.

Plants in RS-supplemented soil could not be accurately measured because of the many fragmented roots.

Values within columns followed by a common letter are not significantly different (P = 0.05).

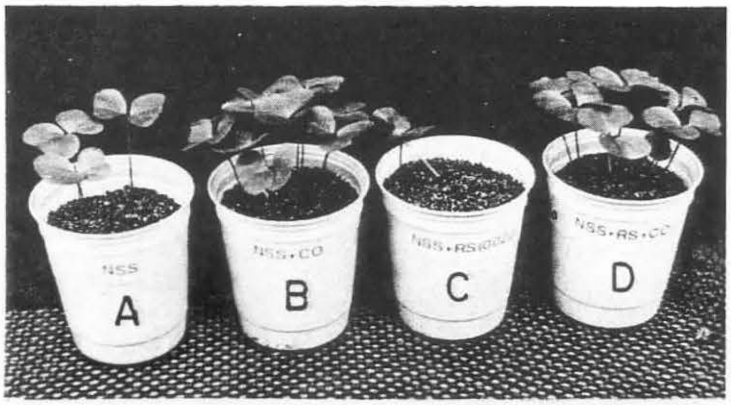

Fig. 3 Control of cotton-seedling disease by 2000 Collembola/kg of non-sterilized field soil supplemented with ground-oat inoculum of *Rhizoctonia solani* at 0.02 g/kg; A) Non-sterilized unamended soil, B) Collembola only, C) *Rh. solani* only, D) *Rh. solani* + Collembola.

healthier and more vigorous. An average increase of 1.3 cm total plant length occurred in naturally RS-infested soil with the double number of Collembola. Length of plants from RS-supplemented soil could not be accurately measured because of many fragmented roots.

Along with the Collembola-imposed benefits to seedling emergence and subsequent growth, severity of root disease was reduced in treatments with added insects (Table 4) The moderate disease severity in naturally RS-infested soil (NSS) was virtually eliminated in the presence of either population of insects. In RS-supplemented soil (NSS+RS+CO), the added benefit of an increased population of insects from 1000 to 2000 was evident as the disease index value was reduced by nearly 50% (4.1 to 2.2). These differences are illustrated in Figure 4. The moderate amount of disease that occurred in unamended natural soil (see Figure 4A) was reduced to almost imperceptible damage where Collembola were added (see Figure 4B); the rotted cotton seeds represent the relative extent of pre-emergence damping-off. Increased root-disease severity and pre-emergence rot where supplementary RS was added without insects can be seen in Figure 4C. Most of these plants were diseased and *Rh. solani* was readily isolated from all affected plants; *Pythium* sp. was isolated only occasionally. Figure 4D shows the benefit of adding Collembola along with the *Rhizoctonia* inoculum. In treatments with Collembola, roots consistently had a cleaner, whiter appearance, and whole root systems were more fully developed.

Discussion

The ecology of soil-inhabiting microarthropods has been studied in great detail, largely in grassland and forest

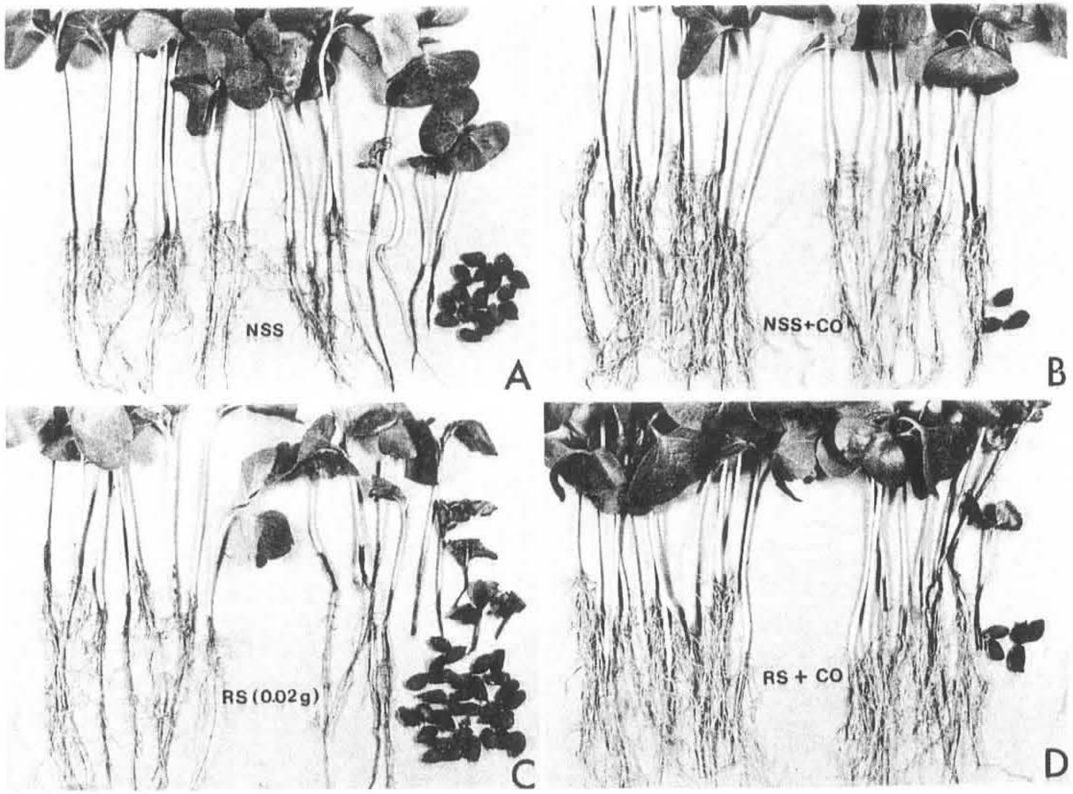

Fig. 4 Cotton seedlings grown in non-sterilized field soil with supplementary *Rhizoctonia* inoculum and Collembola either added or omitted; each picture shows, from left to right, a gradation of disease injury from least to most severe, the ungerminated seeds representing the relative amount of pre-emergence damping off. A) Unamended natural soil. B) Natural soil with only Collembola added at 2000/kg soil. C) Only *Rhizoctonia* inoculum added at 0.02 g/kg-soil. D) Both *Rhizoctonia* inoculum and Collembola added.

soils, but little information is available on their relation to plant roots and the associated microflora. The order Collembola includes a large number of genera and species that inhabit soil and feed on a wide variety of organic materials (Wallwork, 1976). Most are regarded as saprophagous and rarely have been known to injure plant roots. Some are primarily mycophagous, their distribution and feeding activities being related to the availability of the microflora (Christiansen, 1964).

Proistoma minuta and *Onychiurus encarpatus*, used in the present study, probably occupy slightly different levels in the soil profile. It is generally considered that species which live near the soil surface are strongly pigmented and have well developed eyes and springing organs (furcula), as in the case of *Proisotoma*. Deeper soil dwellers tend to have a reduced furcula and little pigmentation, as in *Onychiurus*, and are relatively tolerant of high CO_2 concentrations. Wiggins *et al.* (1979) revealed that these two species were more abundant within the influence of cotton root systems than distant from the rhizosphere. It was then established that *Rhizoctonia* was a favourable food source for rearing these Collembola, and, while they were unable to transmit the fungus in sufficient quantity to initiate disease, it was suggested that they may be a factor in reducing inoculum density (Wiggins and Curl, 1979).

The feeding tests in this study further demonstrated the capacity of *P. minuta* and *O. encarpatus* to consume mycelium of *Rh. solani*, while sterile nutrient agar and organic matter (oats) provided little or no attraction. When the mixed species were applied to dishes of agar with *Rh. solani* and a sterile substrate, they began to move within a few minutes toward the fungus, strongly suggesting directional attraction rather than chance encounter of food source. In tests where the species were applied separately, there was some indication that *O. encarpatus* may be the more destructive feeder. When a common soil saprophyte, *T. harzianum*, was placed in dishes along with *Rh. solani*, the applied insects showed a preference for the latter and abandoned *Trichoderma* almost entirely when sporulation began. This relates to our findings (Wiggins and Curl, 1979) that reproduction of Collembola was suppressed in soil with *T. harzianum* , as well as with abundantly sporulating species of *Penicillium* and *Aspergillus*.

Other observations not emphasized in the Results section of this paper are of interest. Collembola applied to older cultures of *Rh. solani* (after 36 h growth) were not able to consume the entire colony; mycelial aggregations and sclerotia were left behind as the insects tended to congregate around developing hyphal tips. In *T. harzianum* cultures, feeding activity was concentrated largely at the colony periphery where conidia were not yet formed.

In the foregoing tests, water agar was used as the base for substrate and insect application to reduce contamination by bacteria, which are a part of the normal gut flora. Other tests (not reported) were performed in which substrates were placed on the surface of non-sterilized soil. The activities of added Collembola were quite similar to those observed on agar, thus indicating that the insects are not deterred by the normal soil microflora in their movement to and feeding upon *Rh. solani*.

The laboratory tests with sterilized soil showed that Collembola, presumably by destructively altering the inoculum density of *Rh. solani*, can reduce disease severity in cotton seedlings. This occurred in soil with inoculum densities sufficiently high to kill over 90% of the seedlings in the absence of Collembola. A clear relation was shown between the size of insect population and the extent of disease reduction, though further study of this relationship is needed with more precisely controlled numbers. The insect populations, as well as inoculum densities, used in these sterile-soil tests probably are somewhat higher than would be expected to occur in natural cultivated soil.

In three of the four tests with sterile soil, plant growth was enhanced in soil receiving only Collembola; the largest increase in total plant length (root + shoot) was 3.8 cm. This was observed in a previous study (Wiggins and Curl, 1979) in which it was pointed out that Collembola, though reared for several generations on pure cultures of *Rhizoctonia*, are not free of other micro-organisms. Thus, the root surface may become colonized by transported bacteria and perhaps these could increase nutrient or growth-factor availability and uptake (Barber, 1968; Rovira and Davey, 1974). Another possibility in view of the work of Rovira and Bowen (1966), is that plant stimulation could be due to microbial destruction of plant-inhibiting toxins formed by heat sterilization of the soil used. It is unlikely that a mycorrhizal relationship was established under the experimental conditions and short (12-day) growth period involved.

Results of greenhouse experiments in non-sterilized field soil, with its natural organic matter and microflora, seem to confirm the proposal that Collembola may act suppressively upon *Rh. solani* even when other food sources are available. This action contributed to a significant reduction in both pre- and post-emergence damping-off of cotton seedlings, consequently favouring plant growth and a more fully developed root system. Though it is not possible at this time to compare accurately the Collembola populations used in this investigation with the relative numbers reported by Wiggins *et al.* (1979) in the rhizosphere of field cotton, certain facts bear on the subject. A precise method has not been devised for sampling the true rhizosphere for rapidly moving micro-animals; the conventional soil-sampling tubes used by Wiggins *et al.* provided only a partial rhizosphere soil as compared with soil entirely free of roots. Therefore, our previously reported highest population of *P. minuta* and *O. encarpatus* (511/litre of soil), taken from the same sandy loam plot used in the present study, probably was much lower than the actual rhizosphere population, and losses during transportation and extraction of soil samples could have further reduced the true count.

In the present greenhouse study, *Rhizoctonia* inoculum was evenly distributed throughout pots of soil, affording the chance for wide dispersal of added insects as they moved to feeding sites. While it is quite probable that the majority concentrated on the top 3-4 inches of soil around the cotton seed and hypocotyls, many would be expected to migrate to other microflora-colonized particles. From the foregoing considerations, it would seem logical to believe that the Collembola populations used probably were not in excess of numbers which might be expected to occur in the rhizosphere of cotton seedlings under natural conditions in a sandy loam in the field.

Results of this study invite speculation on certain questions:

1) Do micro-arthropods, especially Collembola, contribute significantly to natural suppression of *Rh. solani* and probably other root-infecting fungi under field conditions?
2) Are their numbers promoted to significant levels by organic amendments and cultural practices such as crop rotation with fibrous root-system crops?
3) May Collembola have a natural interacting role with the rhizosphere microflora, contributing to a phase of biological control not previously realized?

Recalling the definition of biological control proposed by Garrett (1970) and the unified concept as stated by Baker and Cook (1974), in which one objective is reduction of inoculum or decreased spread by mycelial growth, it would seem that the micro-arthropod fauna has been neglected. There is a need to expand our concept of the term antagonism to include in a more positive manner the exploitation of fungi by the microfauna in general and Collembola in particular.

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